

10/753,644

45

11. Fmoc-Tyr(^tBu)
to provide 10 mg of N-Ac-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH₂.

EXAMPLE 6

N-Ac-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH₂

5 The title compound was prepared using the synthetic sequence described in Example 1 and using Fmoc-Tyr(^tBu) as amino acid No. 1. The following amino acids were added using the conditions indicated:

No. Amino Acid
2. Fmoc-Asp(β-O^tBu)
3. Fmoc-Tyr(^tBu)
4. Fmoc-Leu
5. Fmoc-Lys(Boc)
6. Fmoc-Arg(Pmc)
7. Fmoc-Pro

to provide N-Ac-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH₂ (4 mg). MS (FAB) m/z 995 (M+H)⁺.

10

EXAMPLE 7

N-Ac-Pro-Arg-Lys-Leu-Tyr-Asp-NH₂

The title compound was prepared using the synthetic sequence described in Example 1. The following amino acids were added using the conditions indicated:

No. Amino Acid
2. Fmoc-Tyr(^tBu)
3. Fmoc-Leu
4. Fmoc-Lys(Boc)
5. Fmoc-Arg(Pmc)
6. Fmoc-Leu

15 to provide N-Ac-Pro-Arg-Lys-Leu-Tyr-Asp-NH₂ (6 mg). MS (ESI) m/z 832 (M+H)⁺.

EXAMPLE 8

Line 18 → N-Ac-Pro-Glu-Lys-Arg-Tyr-Asp-Tyr-NH₂ (SEQ ID NO:39)

20 The title compound was prepared using the synthetic sequence described in Example 1 and using Fmoc-Tyr(^tBu) as amino acid No. 1. The following amino acids were added using the conditions indicated:

No. Amino Acid
2. Fmoc-Asp(β-O^tBu)

Please REPLACE the paragraph at page 4, line 29 with the following amended paragraph:

[FIG. 3] Figures 3(a) and 3(b) show[s] the DNA sequence (SEQ ID NO:12) of human plasminogen.

Please REPLACE the paragraph at page 8, line 18 with the following replacement paragraph:

~~A-Pro-Glu-Lys-Arg-Tyr-Asp-Tyr-Y (SEQ ID NO:39)~~

incorrect

Please REPLACE the paragraph on page 46, line 12 with the following amended paragraph:

N-Ac-Pro-Arg-Lys-Leu-3-I-Tyr-Asp-Tyr-NH₂ (SEQ ID NO:[13]6)

Please REPLACE the paragraph on page 47, line 1 with the following amended paragraph:

N-Ac-Pro-Arg-Lys-Leu-Tyr-Asp-3-I-Tyr-NH₂ (SEQ ID NO:[14]18)

Please REPLACE the paragraph on page 47, lines 17-19 with the following amended paragraph:

Preparation and separation of a mixture N-Ac-Pro-Arg-Lys-Leu-Tyr-Asp-3-I¹²⁵-Tyr⁵³⁵-NH₂ and N-Ac-Pro-Arg-Lys-Leu-3-I¹²⁵-Tyr⁵³³-Asp-Tyr-NH₂ (SEQ ID NO:[13]6) and (SEQ ID NO:[14]18) respectively.

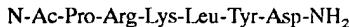
Please REPLACE the paragraph on page 50, lines 4-12 with the following amended paragraph:

The effect of kringle 5 peptide fragments on endothelial cell proliferation was determined *in vitro* using the above described endothelial cell proliferation assay. For these experiments, kringle 5 peptide fragments [was] were prepared as illustrated in Examples 1 through 14 and tested at various concentrations ranging from about 100 to 1000 pM with bFGF used as a maximum proliferation control. The kringle 5 peptide

Error

37

EXAMPLE 7



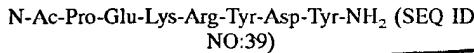
The title compound was prepared using the synthetic sequence described in Example 1. The following amino acids were added using the conditions indicated:

No. Amino Acid

2. Fmoc-Tyr('Bu)
3. Fmoc-Leu
4. Fmoc-Lys(Boc)
5. Fmoc-Arg(Pmc)
6. Fmoc-Leu

to provide N-Ac-Pro-Arg-Lys-Leu-Tyr-Asp-NH₂ (6 mg). MS (ESI) m/z 832 (M+H)⁺.

EXAMPLE 8



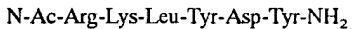
The title compound was prepared using the synthetic sequence described in Example 1 and using Fmoc-Tyr('Bu) as amino acid No. 1. The following amino acids were added using the conditions indicated:

No. Amino Acid

2. Fmoc-Asp(β-O'Bu)
3. Fmoc-Tyr('Bu)
4. Fmoc-Arg(Pmc)
5. Fmoc-Lys(Boc)
6. Fmoc-Glu
7. Fmoc-Pro

to provide N-Ac-Pro-Glu-Lys-Arg-Tyr-Asp-Tyr-NH₂ (6 mg). MS (FAB) m/z (1101) (M+H)⁺.

EXAMPLE 9



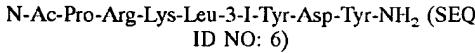
The title compound was prepared using the synthetic sequence described in Example 1 and using Fmoc-Tyr('Bu) as amino acid No. 1. The following amino acids were added using the conditions indicated:

No. Amino Acid

2. Fmoc-Asp(β-O'Bu)
3. Fmoc-Tyr('Bu)
4. Fmoc-Leu
5. Fmoc-Lys(Boc)
6. Fmoc-Arg(Pmc)

to provide N-Ac-Arg-Lys-Leu-Tyr-Asp-Tyr-NH₂ (8 mg). MS (ESI) m/z 898 (M+H)⁺.

EXAMPLE 10



The title compound was prepared using the synthetic sequence described in Example 1 and using Fmoc-Tyr('Bu) as amino acid No. 1. The following amino acids were added using the conditions indicated:

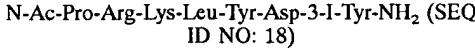
No. Amino Acid

2. Fmoc-Asp(β-O'Bu)
3. Fmoc-3-I-Tyr('Bu)
4. Fmoc-Leu

5. Fmoc-Lys(Boc)
6. Fmoc-Arg(Pmc)
7. Fmoc-Pro
to provide N-Ac-Pro-Arg-Lys-Leu-3-I-Tyr-Asp-Tyr-NH₂ (2 mg). MS (ESI) m/z (1121) (M+H)⁺.

38

EXAMPLE 11

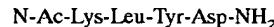


10 The title compound was prepared using the synthetic sequence described in Example 1 and using Fmoc-3-I-Tyr ('Bu) as amino acid No. 1. The following amino acids were added using the conditions indicated:

No. Amino Acid
2. Fmoc-Asp(β-O'Bu)
3. Fmoc-Tyr('Bu)
4. Fmoc-Leu
5. Fmoc-Lys(Boc)
6. Fmoc-Arg(Pmc)
7. Fmoc-Pro

to provide N-Ac-Pro-Arg-Lys-Leu-Tyr-Asp-3-I-Tyr-NH₂ (2.5 mg). MS (ESI) m/z 1121 (M+H)⁺.

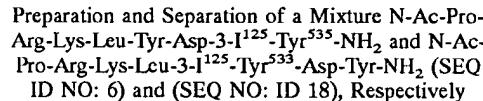
EXAMPLE 12



The title compound was prepared using the synthetic sequence described in Example 1 and using Fmoc-Asp(β-O'Bu) as amino acid No. 1. The following amino acids were added using the conditions indicated:

30 No. Amino Acid
2. Fmoc-Tyr('Bu)
3. Fmoc-Leu
4. Fmoc-Lys
to provide 2 mg of N-Ac-Lys-Leu-Tyr-Asp-NH₂ (2 mg).

EXAMPLE 13

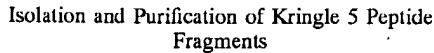


To a solution of 30 µg of N-acetyl-prolyl-arginyl-lysyl-leucyl-tyrosyl-aspartyl-tyrosylamide in 80 mL of phosphate buffered saline (PBS) was added one iodobead (Pierce, Rockford, Ill.) and 100 µCi of NaI¹²⁵. After 10 minutes, the excess NaI¹²⁵ reagent was removed by applying the reaction mixture to a Waters C18-Light SepPack column and eluting with water then 0.1% TFA in 1:1 CH₃CN/water and collecting 3×200 µL fractions to provide a mixture of Tyr⁵³³- and Tyr⁵³⁵-radiolabeled peptides.

50 The hot peptide mixture was co-injected onto a C18 HPLC column with an equimolar solution of cold carriers N-Ac-Pro-Arg-Lys-Leu-Tyr-Asp-3-I-Tyr-NH₂ and N-Ac-Pro-Arg-Lys-Leu-3-I-Tyr-Asp-Tyr-NH₂, the elution times of which had been predetermined as 36 and 38 minutes, respectively. Repeated elutions with the solvent system in Example 1 and lyophilization of the combined, relevant fractions provided the desired compound N-Ac-Pro-Arg-Lys-Leu-Tyr-Asp-3-I-Tyr-NH₂ with a minimal impurity N-Ac-Pro-Arg-Lys-Leu-3-I-Tyr-Asp-Tyr-NH₂.

General Methodologies

EXAMPLE 14



65 The kringle 5 peptide fragments were prepared from the digestion of Lys plasminogen (Lys-HPg, Abbott